

MediGene Aktiengesellschaft

26 January 2001

M29255 BÖ/ATe
PCUS**PATENT CLAIMS**

5

1. The use of a structural protein of adeno-associated virus (AAV) for purifying AAV and/or AAV particles, characterized in that the structural protein comprises at least one mutation which brings about an alteration in the chromatographic properties of the virus.

10

2. The use of a structural protein as claimed in claim 1, characterized in that the alteration in the chromatographic properties makes an improvement in the purification possible, in particular a concentration of the virus, preferably of the virus particles, to higher titers, a purification to greater purity and/or a more efficient purification.

15

20

3. The use of a structural protein as claimed in either of claims 1 or 2, characterized in that the mutation brings about a negligible reduction in the infectivity of the virus.

25

4. The use of a structural protein as claimed in any of claims 1 to 3, characterized in that the mutated structural protein is capable of particle formation.

30

5. The use of a structural protein as claimed in any of claims 1 to 4, characterized in that the mutated structural protein increases the thermal stability.

35

6. The use of a structural protein as claimed in any

of claims 1 to 5, characterized in that it is selected from mutated VP1, mutated VP2 and/or mutated VP3.

5 7. The use of a structural protein as claimed in any of claims 1 to 6, characterized in that it is derived from AAV1, AAV2, AAV3, AAV4, AAV5 and/or AAV6 and other AAV serotypes derived therefrom, in particular from AAV2.

10

8. The use of a structural protein as claimed in any of claims 1 to 7, characterized in that the mutation is a point mutation, a mutation of more than one amino acid, one or more deletion(s), in particular one or more insertion(s) or a combination of said modifications.

15

9. The use of a structural protein as claimed in any of claims 1 to 8, characterized in that amino acids of a functional sequence which are preferably suitable for affinity chromatography are inserted.

20

10. The use of a structural protein as claimed in claim 9, characterized in that the inserted amino acid sequence is selected from a ligand of a receptor or the receptor of a ligand, an antibody or part of an antibody, in particular an antibody epitope, an antigen or antigen epitope, a hormone, a hormone receptor, an enzyme, an enzyme substrate, a lectin, sugar-bearing amino acids, in particular from a histidine-rich peptide (His tag), a multiply charged peptide, glutathione S-transferase (GST tag), an F_c part of an antibody, an immunoglobulin-binding domain, for example protein A or protein G or a part thereof, a lecitin, a nucleic acid binding site, a heparin

25

30

35

- binding site, a specific ligand, a specific receptor, an integrin, a cytokine or a receptor binding domain of a cytokine, integrin or growth factor, single-chain antibodies which bind to a cell surface receptor, an antibody against cell surface structures, an epitope and/or an antibody-binding structure.
11. The use of a structural protein as claimed in either of claims 9 or 10, characterized in that a peptide which has the sequence QAGTFALRGDNPQG is inserted.
12. The use of a structural protein as claimed in any of claims 1 to 11, characterized in that the structural protein comprises at least one other mutation.
13. The use of a structural protein as claimed in claim 12, characterized in that the other mutation(s) brings about an alteration in the infectivity of the virus.
14. The use of a structural protein as claimed in either of claims 12 or 13, characterized in that the other mutation(s) brings about a reduction in the antigenicity of the virus.
15. The use of a structural protein as claimed in any of claims 12 to 14, characterized in that the other mutation(s) is/are one or more deletion(s), one or more insertion(s) or a combination of said modifications.
16. The use of a structural protein as claimed in any of claims 12 to 15, characterized in that the insertion is a cell membrane receptor ligand, a

Rep protein or peptide, an immunosuppressive protein or peptide and/or a protein or peptide with a signal for double strand synthesis of the foreign gene.

5

17. The use of a structural protein as claimed in any of claims 12 to 16, characterized in that the insertion is selected from an integrin, a cytokine or a receptor binding domain of a cytokine, integrin or growth factor, single-chain antibodies which bind to a cell surface receptor, an antibody against cell surface structures, an antibody-binding structure or an epitope.

10

18. The use of a structural protein as claimed in any of claims 1 to 17, characterized in that the mutation(s) is/are located on the virus surface.

15

19. The use of a structural protein as claimed in any of claims 1 to 18, characterized in that the mutation(s) is/are located at the N terminus of the structural protein.

20

20. The use of a structural protein as claimed in any of claims 1 to 19, characterized in that the mutation(s) is/are brought about by one or more insertions in the XhoI cleavage site of the VP1-encoding nucleic acid.

25

21. The use of a structural protein as claimed in any of claims 1 to 20, characterized in that the mutation(s) is/are brought about by one or more insertions in the BsrBI cleavage site of the VP1-encoding nucleic acid.

30

35

22. The use of a structural protein as claimed in any of claims 1 to 21, characterized in that the

mutation(s) is/are brought about by one or more deletions between the BsrBI-HindII cleavage sites of the VP1-encoding nucleic acid and one or more insertions.

5

23. The use of a structural protein as claimed in any of claims 1 to 22, characterized in that the mutation(s) is/are brought about by one or more deletions between the XhoI-XhoI cleavage sites of the VP1-encoding nucleic acid.

10

24. The use of a structural protein as claimed in any of claims 1 to 23, characterized in that the mutation(s) is/are brought about by one or more deletions between the BsrBI-HindII cleavage sites of the VP1-encoding nucleic acid.

15

25. The use of a structural protein as claimed in any of claims 1 to 19, characterized in that one or more insertions in VP3 is/are located before and/or after at least one amino acid in the sequence selected from YKQIS SQSGA, YLTLN NGSQA, YYLSR TNTPS, EEKFF PQSGV, NPVAT EQYGS, LQRGN RQAAT, NVDFV VDTNG.

20

25

26. The use of a structural protein as claimed in any of claims 1 to 25 in the form of an AAV particle, in particular in the form of an AAV capsid.